National Institute of Environmental Health Sciences

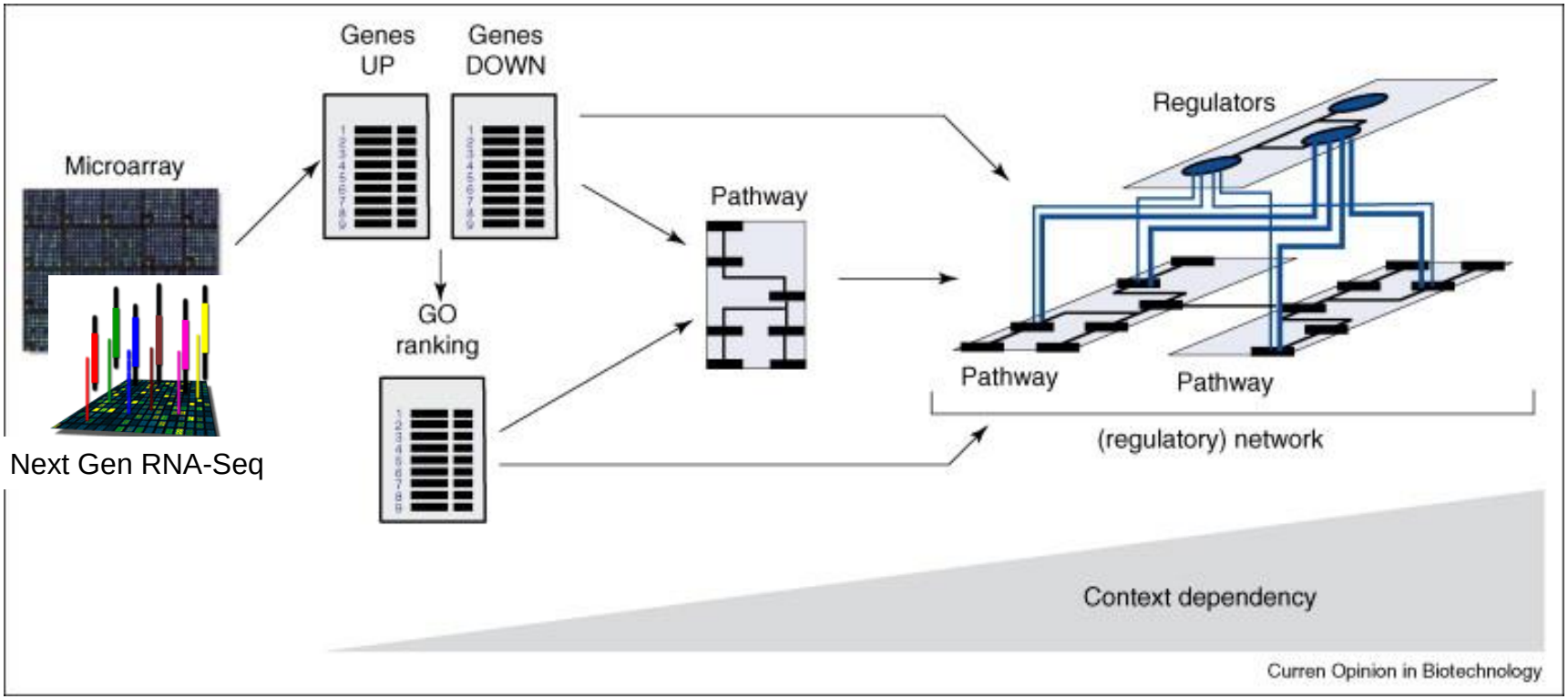
Your Environment. Your Health.

Pathway analysis

Biostat & Bioinfo short course series

Jianying Li

The Road to Pathway Analysis



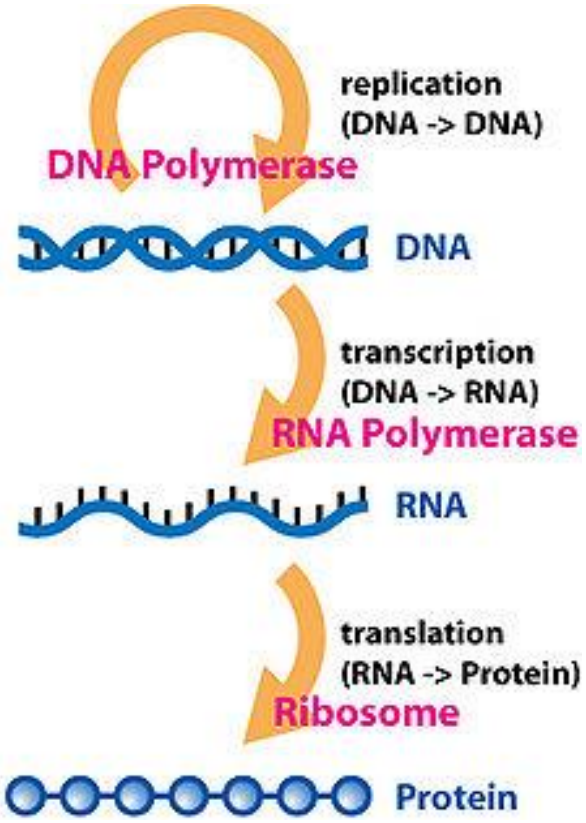
Adapted from Werner (2008), Current Opinion in Biotech., 19:50-64

Outline of the pathway analysis

- Knowledge based pathway analysis
  - Available knowledge bases
- Two major statistical approaches
  - Parametric: Hypergeometric/Fisher Exact Test (FET)
  - Non-parametric: Kolmogorov-Smirnov/ranking statistics
- Hands on practice
  - Parametric --> IPA
  - Non-parametric --> GSEA
- The proceedings in pathway analysis research

What do we mean by pathway?

Central Dogma



Involvement of Gene Products

- Biological process or molecular function
- Signaling cascades
- Etc.
- Genes are categorized based on criteria

Gene sets (e.g. function driven)

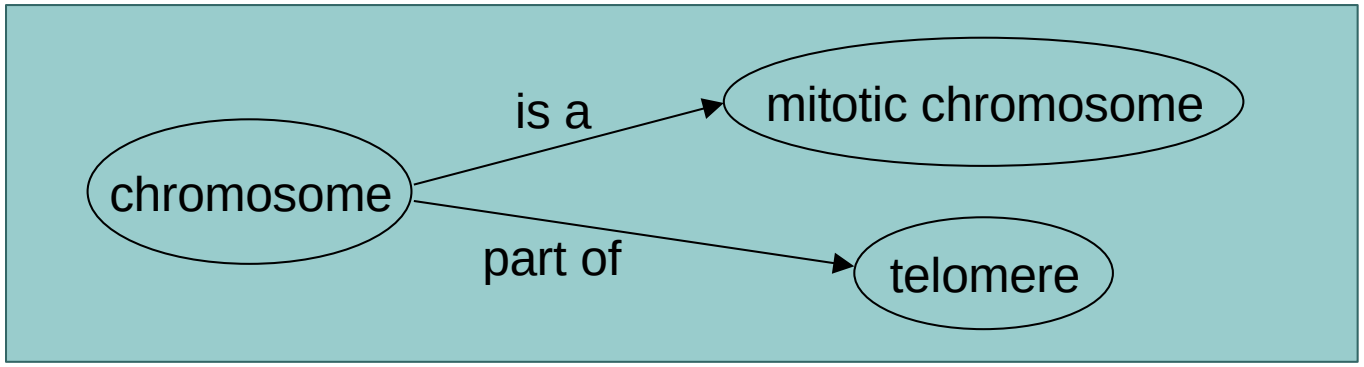
- Genes (sets) have something in common
  - On the same cytogenetic band
  - Coding for proteins that are part of the same cellular component
  - Can be part of the same biochemical pathway
  - Co-expressed under certain conditions
  - Putative targets of the same regulatory factor
  - ….

Gene Ontology

- **Gene Ontology (GO)** Consortium was established in 1998 to developed shared, structured vocabulary (an ontology) for the annotation of molecular characteristics across different organisms.
  - a collaborative effort to address the need for consistent descriptions of gene and gene products in different databases
  - Original members of the consortium: SGD, FlyBase and MGD
- Two primary purposes for an ontology:
  1. to facilitate communication between people and organizations
  2. to improve upon the interoperability between systems

GO structure

- The ontologies are structured vocabularies in the form of directed acyclic graphs (DAGs)
- The DAG represents a network (not a tree) in which each term may be a child of one or more than one parent
- The relationships of child to parent can be of the “is a” type or the “part of” type



Ontologies within GO

- molecular function describing activities, such as catalytic or binding activities, at the molecular level
- biological process referring to a biological objective to which the gene product contributes
- cellular component referring to the place in the cell (i.e. the location) where a gene product is found

```
[Term]
id: GO:0004363
name: glutathione synthase activity
namespace: molecular_function
def: "Catalysis of the reaction: L-gamma-glutamyl-L-cysteine + ATP + glycine = ADP + glutathione + 2 H(+) + phosphate."
subset: gosubset_prok
synonym: "gamma-L-glutamyl-L-cysteine:glycine ligase (ADP-forming)" EXACT [EC:6.3.2.3]
synonym: "glutathione synthetase activity" EXACT [EC:6.3.2.3]
synonym: "GSH synthetase activity" EXACT [EC:6.3.2.3]
xref: EC:6.3.2.3
xref: KEGG:R00497
xref: MetaCyc:GLUTATHIONE-SYN-RXN
xref: Reactome:REACT_100394 "gamma-glutamylcysteine combines with glycine to form glutathione, Caenorhabditis elegans"
xref: Reactome:REACT_104397 "gamma-glutamylcysteine combines with glycine to form glutathione, Saccharomyces cerevisiae
xref: Reactome:REACT_105605 "gamma-glutamylcysteine combines with glycine to form glutathione, Bos taurus"
xref: Reactome:REACT_105891 "gamma-glutamylcysteine combines with glycine to form glutathione, Taeniopygia guttata"
xref: Reactome:REACT_108106 "gamma-glutamylcysteine combines with glycine to form glutathione, Sus scrofa"
xref: Reactome:REACT_108848 "gamma-glutamylcysteine combines with glycine to form glutathione, Rattus norvegicus"
xref: Reactome:REACT_6886 "gamma-glutamylcysteine combines with glycine to form glutathione, Homo sapiens"
xref: Reactome:REACT_80709 "gamma-glutamylcysteine combines with glycine to form glutathione, Arabidopsis thaliana"
xref: Reactome:REACT_81099 "gamma-glutamylcysteine combines with glycine to form glutathione, Canis familiaris"
xref: Reactome:REACT_82957 "gamma-glutamylcysteine combines with glycine to form glutathione, Drosophila melanogaster"
xref: Reactome:REACT_84994 "gamma-glutamylcysteine combines with glycine to form glutathione, Mus musculus"
xref: Reactome:REACT_86625 "gamma-glutamylcysteine combines with glycine to form glutathione, Schizosaccharomyces pombe
xref: Reactome:REACT_86921 "gamma-glutamylcysteine combines with glycine to form glutathione, Oryza sativa"
xref: Reactome:REACT_87951 "gamma-glutamylcysteine combines with glycine to form glutathione, Xenopus tropicalis"
xref: Reactome:REACT_90100 "gamma-glutamylcysteine combines with glycine to form glutathione, Dictyostelium discoideum"
xref: Reactome:REACT_93797 "gamma-glutamylcysteine combines with glycine to form glutathione, Gallus gallus"
xref: Reactome:REACT_99493 "gamma-glutamylcysteine combines with glycine to form glutathione, Danio rerio"
xref: Reactome:REACT_99980 "gamma-glutamylcysteine combines with glycine to form glutathione, Plasmodium falciparum"
xref: RHEA:13560
is_a: GO:0016881 ! acid-amino acid ligase activity
relationship: part_of GO:0006750 ! glutathione biosynthetic process
```

```
[Term]
id: GO:0010778
name: meiotic DNA repair synthesis involved in reciprocal meiotic recombination
namespace: biological_process
def: "The synthesis of DNA proceeding from the broken 3' single-strand DNA end that uses the homologous intact duplex
is_a: GO:0000711 ! meiotic DNA repair synthesis
intersection_of: GO:0000711 ! meiotic DNA repair synthesis
intersection_of: part_of GO:0007131 ! reciprocal meiotic recombination
relationship: part_of GO:0007131 ! reciprocal meiotic recombination

[Term]
id: GO:0010779
name: meiotic DNA repair synthesis involved in meiotic gene conversion
namespace: biological_process
def: "The synthesis of DNA proceeding from the broken 3' single-strand DNA end that uses the homologous intact duplex
is_a: GO:0000711 ! meiotic DNA repair synthesis
intersection_of: GO:0000711 ! meiotic DNA repair synthesis
intersection_of: part_of GO:0006311 ! meiotic gene conversion
relationship: part_of GO:0006311 ! meiotic gene conversion
```

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```
[Term]
id: GO:0038142
name: EGFR:ERBB2 complex
namespace: cellular_component
def: "A heterodimeric complex between the tyrosine kinase receptor ERBB2 and a ligand-activated epidermal growth facto
synonym: "EGF:EGFR:ERBB2 complex" NARROW [Reactome:REACT_116379.1]
synonym: "EGFR:ERBB2 heterodimer" RELATED [Reactome:REACT_116379.1]
xref: Reactome:REACT_116379.1 "ERBB2 heterodimers"
is_a: GO:0043235 ! receptor complex
is_a: GO:0044459 ! plasma membrane part
created_by: rfoulger
creation_date: 2012-03-30T02:10:38Z
```

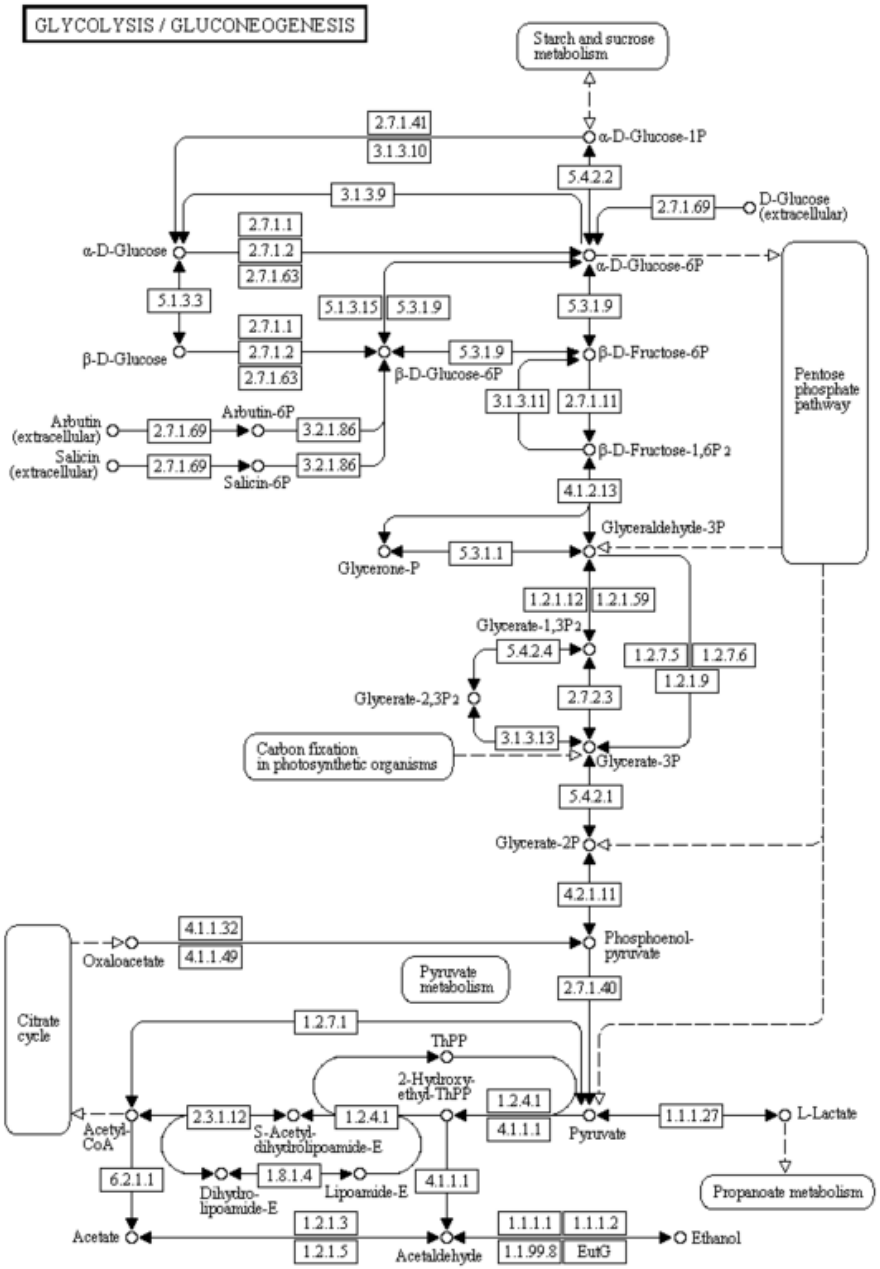
KEGG: Kyoto Encyclopedia of Genes and Genomes

About KEGG

- **Kyoto Encyclopedia of Genes and Genomes (KEGG)** knowledgebase was developed in 1996 consisting of genetic building blocks of genes and proteins.
- A collection of manually drawn pathway maps representing current knowledge on the molecular interaction and reaction networks
- Manually curated based on published literature
- Constructed as wiring diagrams with enzymes and proteins, processes and reactions and substrates, co-factors, intermediates, metabolites and end products

Category in KEGG

- Metabolism: carbohydrates, energy, lipid, nucleotides, amino acid, xenobiotics
- Genetic information processing
- Environmental information processing
- Cellular processes
- Human diseases
- Drug development: the structure relationships


GLYCOLYSIS / GLUCONEOGENESIS
Starch and sucrose metabolism
2.7.1.41
3.1.3.10
α-D-Glucose-1P
5.4.2.2
3.1.3.9
2.7.1.69
D-Glucose (extracellular)
2.7.1.1
2.7.1.2
2.7.1.63
α-D-Glucose
α-D-Glucose-6P
5.1.3.3
5.1.3.15
5.3.1.9
5.3.1.9
2.7.1.1
2.7.1.2
2.7.1.63
β-D-Glucose
5.3.1.9
β-D-Fructose-6P
β-D-Glucose-6P
Pentose phosphate pathway
3.1.3.11
2.7.1.11
Arbutin (extracellular)
2.7.1.69
Arbutin-6P
3.2.1.86
Salicin (extracellular)
2.7.1.69
Salicin-6P
3.2.1.86
β-D-Fructose-1,6P2
4.1.2.13
Glyceraldehyde-3P
Glycerone-P
5.3.1.1
1.2.1.12
1.2.1.59
Glycerate-1,3P2
5.4.2.4
1.2.7.5
1.2.7.6
1.2.1.9
Glycerate-2,3P2
2.7.2.3
3.1.3.13
Carbon fixation in photosynthetic organisms
Glycerate-3P
5.4.2.1
Glycerate-2P
4.2.1.11
4.1.1.32
4.1.1.49
Oxaloacetate
Phosphoenol-pyruvate
Pyruvate metabolism
2.7.1.40
Citrate cycle
1.2.7.1
ThPP
2-Hydroxy-ethyl-ThPP
1.2.4.1
1.2.4.1
4.1.1.1
1.1.1.27
L-Lactate
Pyruvate
2.3.1.12
1.2.4.1
Acetyl-CoA
S-Acetyl-dihydrolipoamide-E
6.2.1.1
1.8.1.4
4.1.1.1
Dihydro-lipoamide-E
Lipoamide-E
Propanoate metabolism
1.2.1.3
1.2.1.5
1.1.1.1
1.1.99.8
1.1.1.2
EutG
Acetate
Acetaldehyde
Ethanol

Transfac and Transpath databases

--Signaling Pathways

Experimentally validated

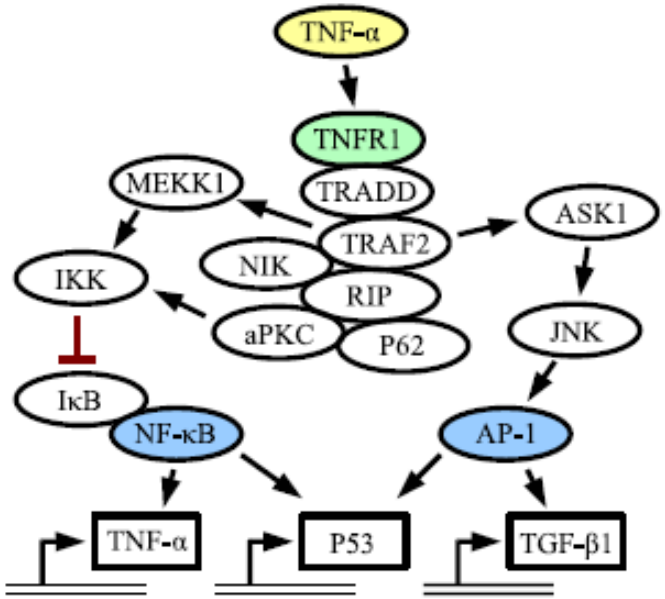


Figure 1
Example pathway. A simplified and partial view of the TNF-α pathway. A ligand (yellow) binds to a receptor (green) on the cell surface, triggering a cascade of events. Eventually, transcription factors (blue) activate or repress the expression of genes.

- **Transfac** - data on transcription factors, their experimentally-proven binding sites, and regulated genes.

  The compilation of binding sites allows the derivation of positional weight matrices.

- **Transpath** – data about protein-protein interactions and directed modification of proteins involved in signal transduction pathways,

  With a particular focus on signaling cascades that affect the activity of transcription factors.

Breslin et al., BMC Bioinformatics, 2005, 6:163

More on gene set collections

- Gene Ontology (GO)
  - Cellular components (CC)
  - Biological processes (BP)
  - Molecular functions (MF)
- Well curated pathway databases
  - KEGG pathway
  - Biocarta
  - GenMAPP
  - IPA pathway database
- Gene set collections
  - MSigDB (GSEA)
  - Gazer
  - Customized collections(GSA)

A parametric approach

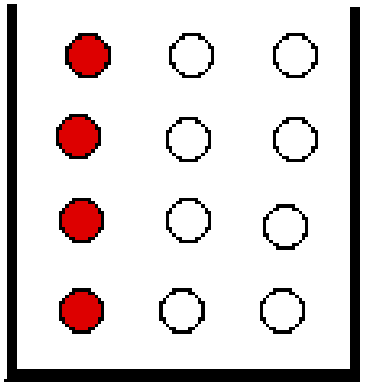
- Hypergenometric distribution and testing
  - A little theoretical background
  - Distribution density
  - Formulating the test
- Fisher's exact test
  - Two by two contingent table
  - One-tailed test formulation
- Watch out for a common error

Hypergeometric Distribution

$$P(X = k) = \frac{\binom{m}{k}\binom{N-m}{n-k}}{\binom{N}{n}}.$$

for k = 0,1,2,…,n
$k<=m$, $n-k<=N-m$

A discrete probability distribution that describes the number of successes (k) in a sequence of n draws from a finite population without replacement



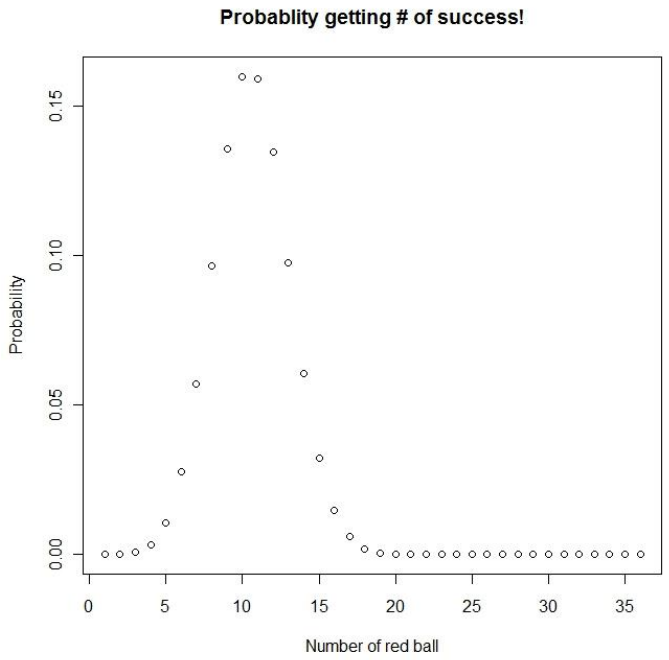
P(k=2, n, m, N) = 0.339

Cumulative dist.:
P(k<=2) = 0.933

- An urn with two types of marbles:
  - **N total # of marbles**
  - **Of which, m # of red marbles**
  - **Drawing a red marble is a success!**
  - **Drawing a white marble is a failure!**
- n is the # of marbles randomly drawn
- k is the # of successes (red marbles) in the sample
- Hypergeometric distribution gives the probability

Hypergeometric distribution

- Number of red ball: 50 ← m
- Number of white ball: 120 } N
- Number of ball drawn (without replacement): 36 ← n
- Possible number of success ??
  - (0,1, 2, ….36),
- Probability to get 20 red balls is: 0.0001494571 p(k=20, 36, 50, 170)



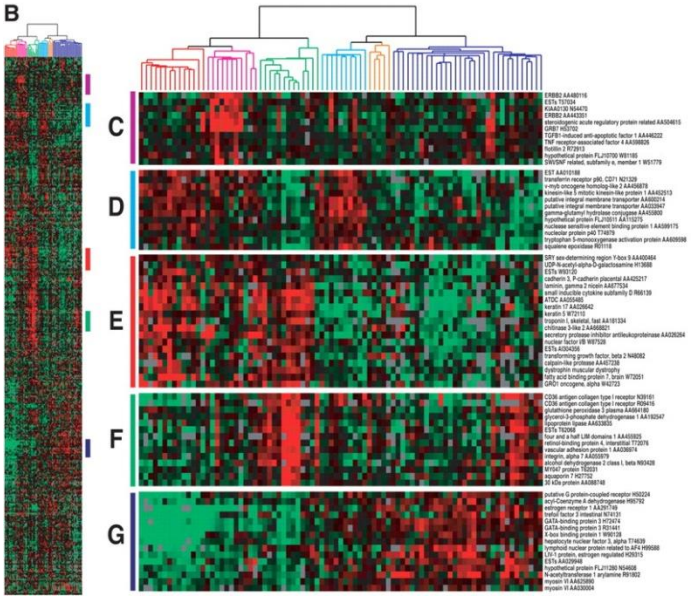
http://www.aiaccess.net/English/Glossaries/GlosMod/e_gm_hypergeometric.htm

Rationale (an analogy)

- A study with gene expression microarray, can be RNAseq also (total # of genes -- **N**)
- Experiment samples from two or more conditions (control vs. treated, wild type vs knock out, etc.)
- Several biological replicates at each condition
- Differentially Expressed Genes (DEGs) obtained from statistical models (total # of genes drawn -- **n**)
- Of which, **k** genes belong to a "**pathway**"
- There are totally **m** genes (out of **N**) belong to this **pathway**
- Is this pathway (the biological process) significantly enriched? (Perturbed? Turned on? Involved?)

Hypergeometric Distribution/Test

in the context of gene expression



$$P(x=k)=\frac{\binom{m}{k}\binom{N-m}{n-k}}{\binom{N}{n}}$$

$$p=1-\sum_{i=0}^{k-1}\frac{\binom{m}{i}\binom{N-m}{n-i}}{\binom{N}{n}}$$

Models the probability the of observing **k** genes from a cluster of **n** genes by chance in a pathway or biological process category containing **m** genes from a total genome (or array) size of **N** genes.

The p-value indicates the probability that the biological process category (particular pathway) is enriched by this microarray experiment by chance. The smaller the p-val, the higher the significance

Pathway analysis

- Gene expression analysis results
  - Total number of gene on a chip: **520**
  - Of which **20** genes are in a GO
  - Total number of DEG: **40**
  - Of which **5** genes are in GO
- Do you think it is significant? In other words "is this GO category enriched by/significant this microarray experiment"?
- The answer is simple, which is to perform a hypergeometric test: ***phyper(4, 20, 500, 40, lower.tail=FALSE) = 0.01371***
- Sometime people report: ***phyper(5, 20, 500, 40, lower.tail=FALSE) = 0.002459***

Watch out!!

Fisher's Exact Test (FET)

--right-tailed test

Contingency table

	DEG	Not DEGs	totals
In a GO category	x	m-x	m
Not in GO category	k-x	n-k+x	n
totals	k	m+n -k	m + n (genes on array)

So, now you are probably given something like the following:

N
- x <- 5 #num_of_DEG in GO
- m <- 20 #num_of_gene on chip in GO
- n <- 500 #num_of_gene on chip NOT in GO
- k <- 40 #num_of_DEG

Comparing hypergeometric test vs. FET, watch out!!

phyper((x-1), m, n, k, lower.tail=FALSE)

(fisher.test(matrix(c(x,(k-x), (m-x), (n-k+x)),2,2), alternative='greater'))$p.value

Ingenuity Pathway Analysis (IPA)

--Knowledgebase

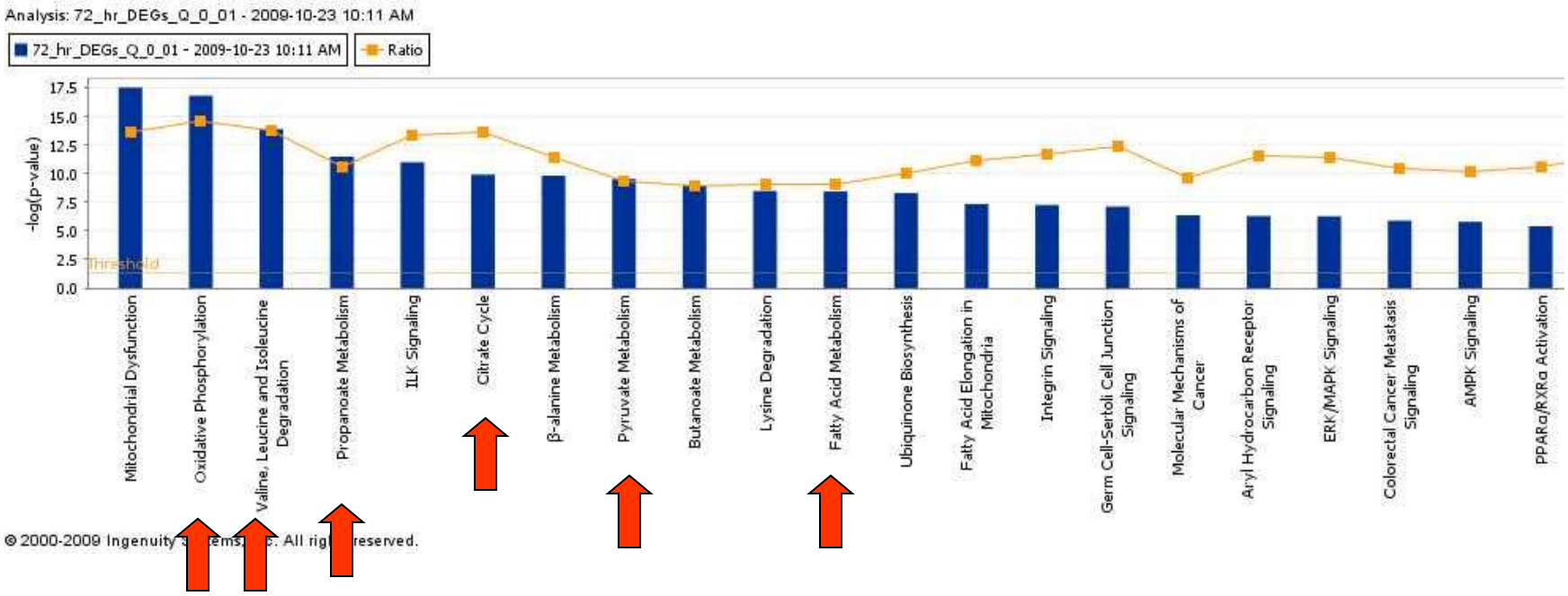
- Desktop Java application utilizing a remote server for data, analysis and file management
- IPA Ontology: Curation of the scientific literature and content extraction of the IPA repository of molecular interactions, regulatory events, biological processes, gene-to-phenotype associations, and chemical knowledge

Ingenuity® Expert Findings	Experimentally demonstrated Findings that are manually curated for accuracy and contextual details from the full-text of articles in top journals.
Ingenuity® ExpertAssist Findings	Manually reviewed, automatically extracted Findings from the abstracts of a broad range of recently published journal articles.
Ingenuity® Expert Knowledge	Knowledge modeled by Ingenuity experts such as pathways, toxicity lists, and more.
Ingenuity® Supported Third Party Information	Manually reviewed content from selected sources and databases such as BIND, Argonaute 2, etc.

IPA Enrichment Analysis

- Uses the Fisher's exact test to determine the significance of a functional group or pathway
  - # molecules in a list that are associated with a function/pathway (**k**)
  - total # of molecules that are associated with a function/pathway (**m**)
  - # of molecules in all possible functions/pathways (**N**)
  - # of molecules in a list (**n**)

IPA Canonical Pathway Over-representation



Canonical pathway: Highly curated metabolic and cell signaling pathways from KEGG, scientific literature

Ratio = # of genes in a pathway that meet cutoff criteria / total # of genes in the pathway

Parametric approach summary

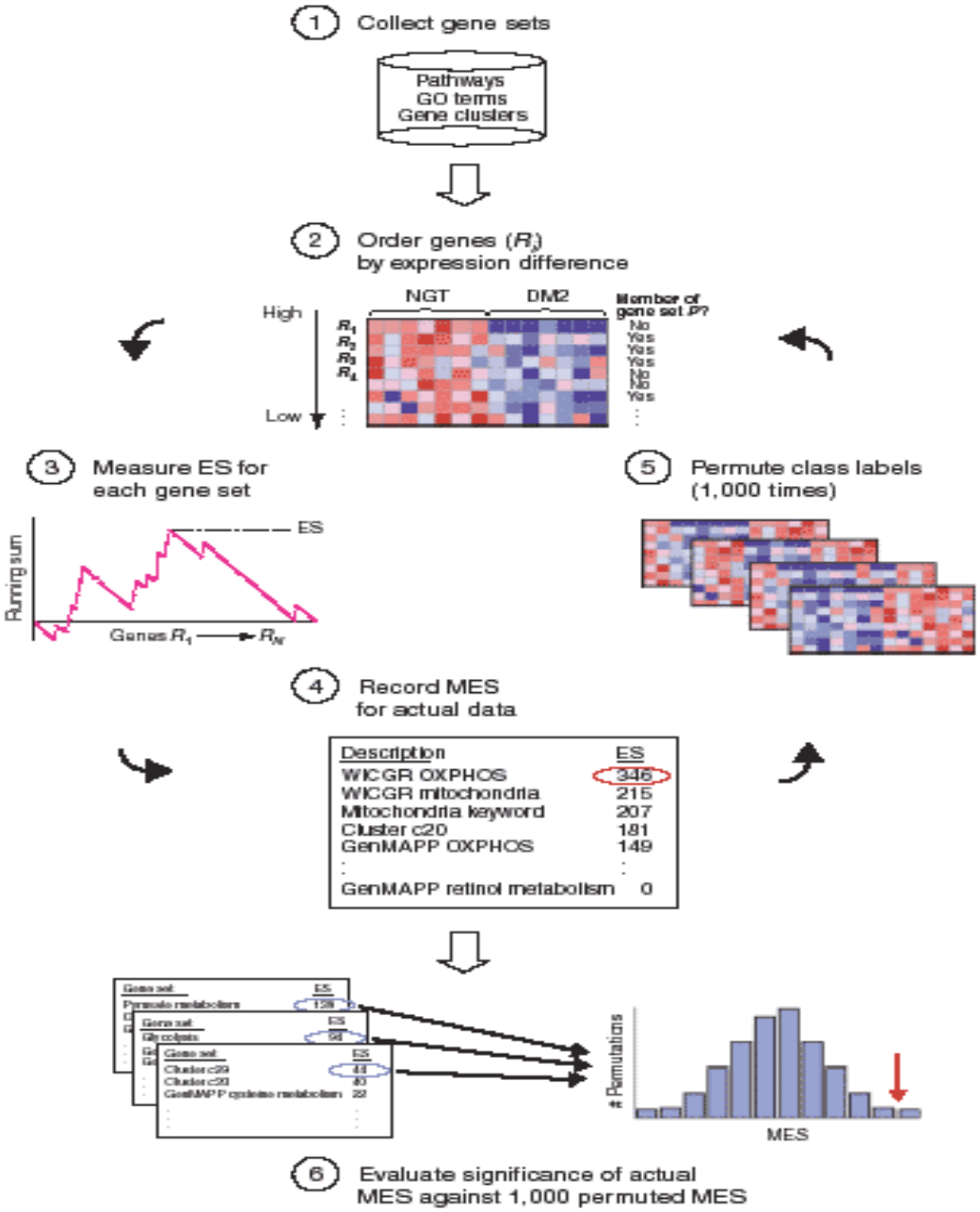
- Statistics distribution based test (hyper geometric/FET)
- And, there are a few violations (will be addressed shortly)
- Knowledgebase used matters
- Hands on practice on IPA

A non-parametric approach

- Based on Kolmogorov-Smirnov (K-S) test
- Compare a sample empirical distribution function to the reference distribution
- Or, compare two sample empirical distribution functions
- The test is performed in relevant to different null hypothesis setting

Gene set enrichment analysis (GSEA)

- Given an *a priori* defined set of genes (i.e. genes encoding products in a metabolic pathway, or sharing the same GO category)
- The goal is to determine whether the members in a set S are randomly distributed throughout all sorted genes (L)
- Mootha *et al.* [2003] suggest to use the Kolmogorov-Smirnov statistic:
  - First, sort all genes by some criteria
  - Go through the list, increasing a running sum for each gene in the gene set by (*N*-*n*)
    - $X_i = \sqrt{\frac{N-G}{G}}$, if R_i is a member of S
  - And decreasing it for each gene not in the gene set by *n*. [*N*: number of genes, *n*: size of gene set]
    - $X_i = -\sqrt{\frac{G}{N-G}}$, if R_i is not a member of S
- The maximum value of the running sum is the enrichment score (ES)
    - $ES = \max_{1 \le j \le N} \sum_{i=1}^{j} X_i$
- Statistical significance determined by permutation GSEA-P

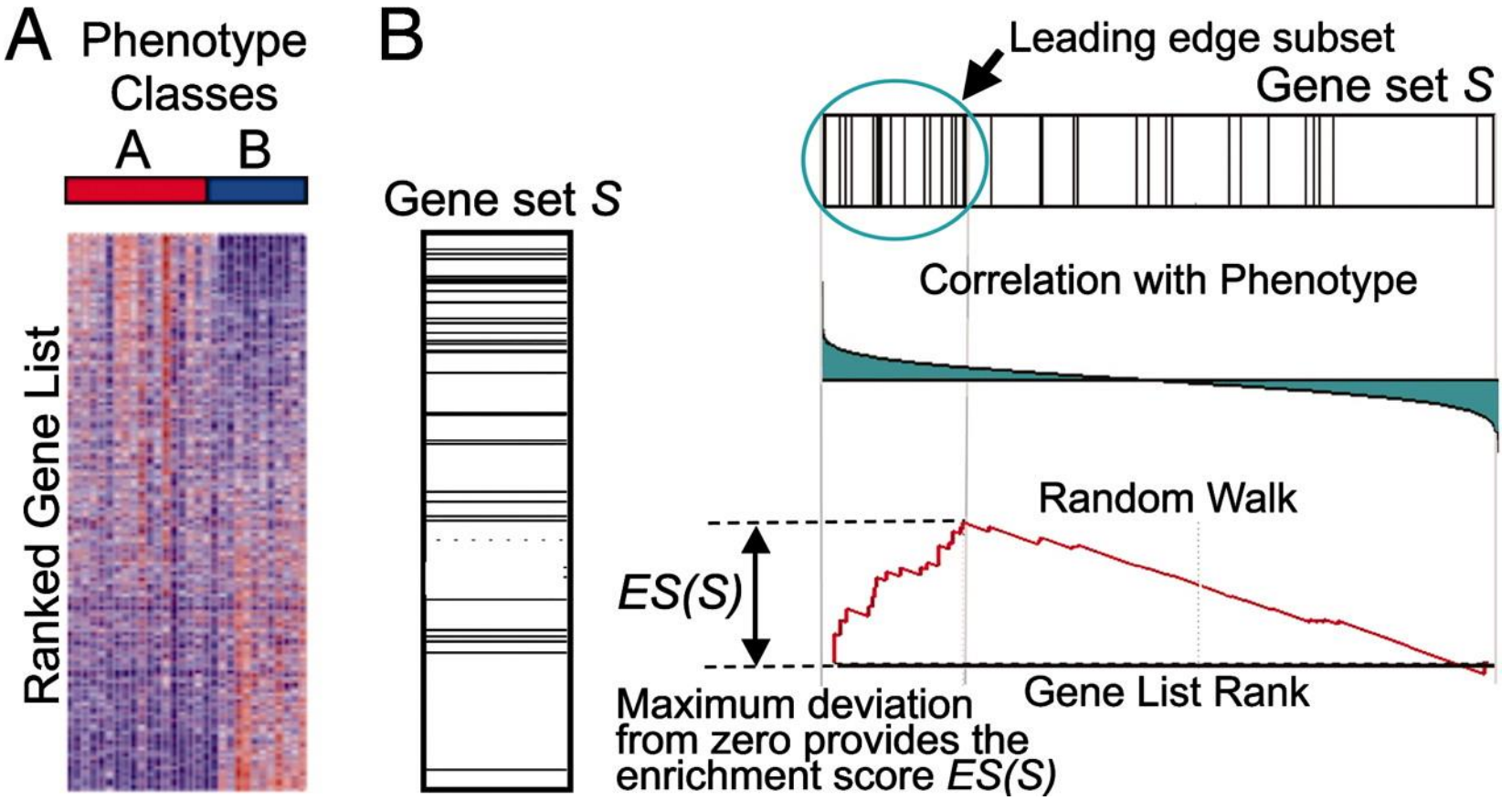


Mootha et al., Nature Genetics, 2003, 34(3):267-273

Improved KS statistics tests

- Subramanian *et al.*, PNAS **102** (2005) proposed an improvement strategy
- $P_{hit}(S,i) = \sum_{\substack{g_{j \in S} \\ j \le i}} \frac{|r_j|^p}{N_R}, where\ N_R = \sum_{g_{j \in S}} |r_j|^p$
- $P_{miss}(S,i) = \sum_{\substack{g_{j! \in S} \\ j \le i}} \frac{1}{(N - N_H)}$
- The ES is the maximum deviation from zero of p_{hit}-p_{miss}
- If p =0, ES reduces to Kolmogorov-Smirnov statistics, in the paper, author used p =1

A GSEA overview illustrating the method.



Subramanian A et al. PNAS 2005;102:15545-15550

GSEA web-portal

http://www.broadinstitute.org/gsea/msigdb/index.jsp

Collections

The MSigDB gene sets are divided into 6 major collections:

c1 **positional gene sets** for each human chromosome and cytogenetic band.

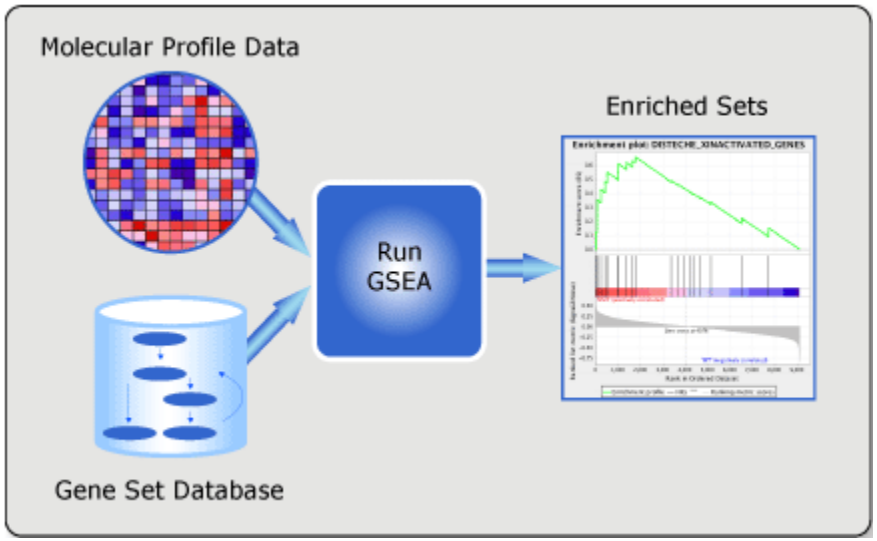
c2 **curated gene sets** from online pathway databases, publications in PubMed, and knowledge of domain experts.

c3 **motif gene sets** based on conserved cis-regulatory motifs from a comparative analysis of the human, mouse, rat, and dog genomes.

c4 **computational gene sets** defined by mining large collections of cancer-oriented microarray data.

c5 **GO gene sets** consist of genes annotated by the same GO terms.

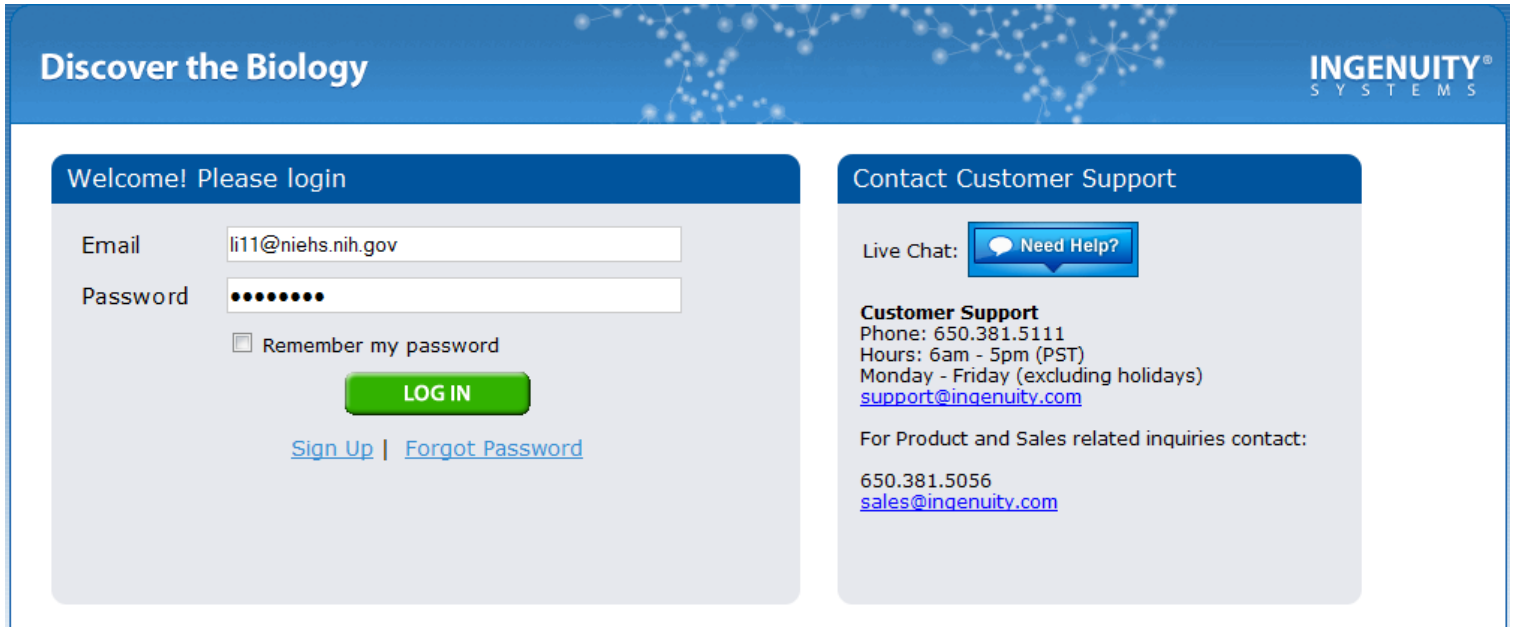
c6 **oncogenic signatures** defined directly from microarray gene expression data from cancer gene perturbations.



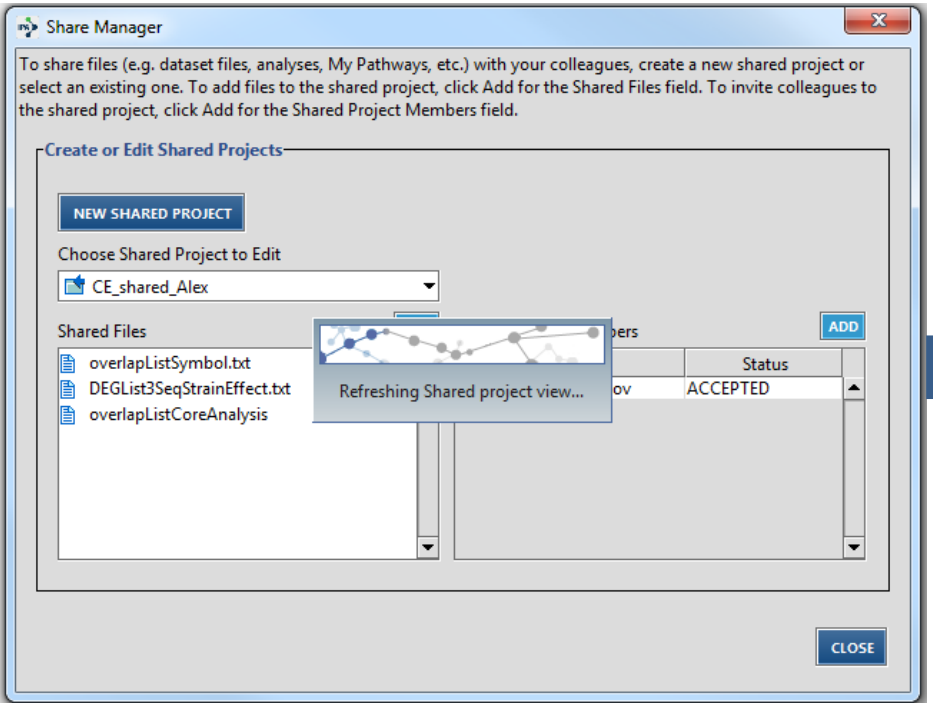
Hands on practice on both parametric and non-parametric

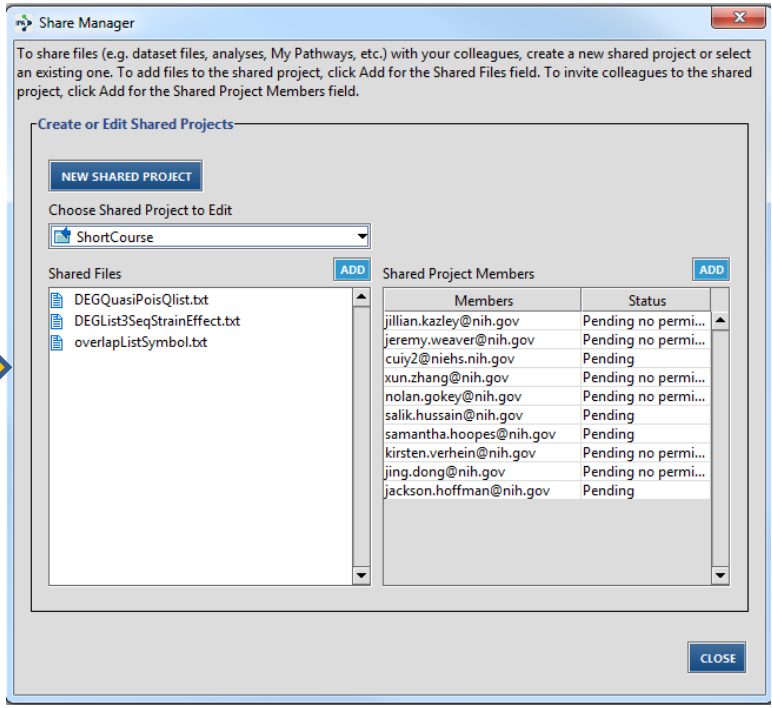
NOW, IT IS YOUR TURN

Ingenuity Pathway Analysis



Sharing a project





Bio function associated -- IPA

Top Bio Functions

Diseases and Disorders

Name	p-value	# Molecules
Inflammatory Response	4.52E-12 - 6.18E-04	106
Infectious Disease	2.80E-07 - 5.75E-04	41
Connective Tissue Disorders	3.01E-07 - 5.03E-05	55
Inflammatory Disease	3.01E-07 - 4.16E-04	72
Skeletal and Muscular Disorders	3.01E-07 - 4.16E-04	56

Molecular and Cellular Functions

Name	p-value	# Molecules
Cell Morphology	2.16E-13 - 7.75E-04	77
Cell Death and Survival	4.69E-12 - 9.35E-04	135
Lipid Metabolism	1.04E-10 - 9.56E-04	89
Small Molecule Biochemistry	1.04E-10 - 9.56E-04	102
Cell-To-Cell Signaling and Interaction	3.11E-10 - 8.78E-04	93

Physiological System Development and Function

Name	p-value	# Molecules
Hematological System Development and Function	1.81E-12 - 9.52E-04	98
Tissue Morphology	1.81E-12 - 8.39E-04	91
Tissue Development	2.38E-12 - 8.87E-04	69
Immune Cell Trafficking	4.52E-12 - 9.52E-04	65
Humoral Immune Response	2.18E-07 - 5.16E-04	38

Other pathways (IPA – cont.)

Top Canonical Pathways

Name	p-value	Ratio
Nicotine Degradation II	3.17E-14	16/83 (0.193)
Nicotine Degradation III	1.53E-11	13/71 (0.183)
LPS/IL-1 Mediated Inhibition of RXR Function	8.06E-10	22/239 (0.092)
Bupropion Degradation	1.28E-09	9/33 (0.273)
Acetone Degradation I (to Methylglyoxal)	1.88E-09	9/36 (0.25)

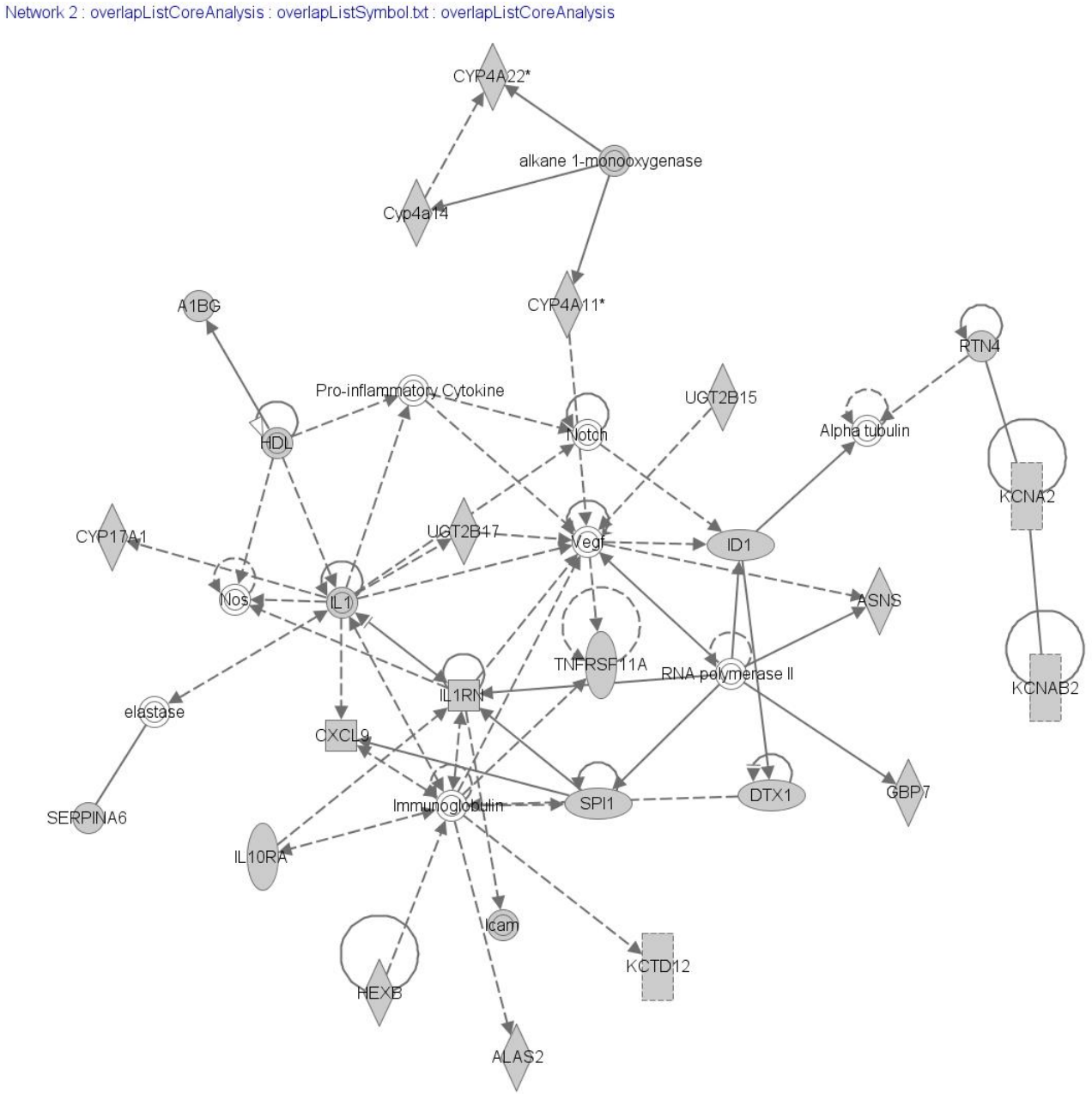
Top Upstream Regulators

Upstream Regulator	p-value of overlap	Predicted Activat.
RORC	3.13E-31	
RORA	2.09E-29	
GPD1	7.95E-23	
SLC25A13	1.45E-22	
NR1I3	5.71E-17	

Top Tox Lists

Name	p-value	Ratio
LPS/IL-1 Mediated Inhibition of RXR Function	1.22E-12	27/247 (0.109)
Cytochrome P450 Panel - Substrate is a Xenobiotic (Mouse)	8.49E-10	9/25 (0.36)
NRF2-mediated Oxidative Stress Response	2.4E-09	22/232 (0.095)
CAR/RXR Activation	3.87E-09	9/29 (0.31)
Cytochrome P450 Panel - Substrate is a Xenobiotic (Rat)	2.22E-08	8/25 (0.32)

Lipid metabolism, endocrine system development and function



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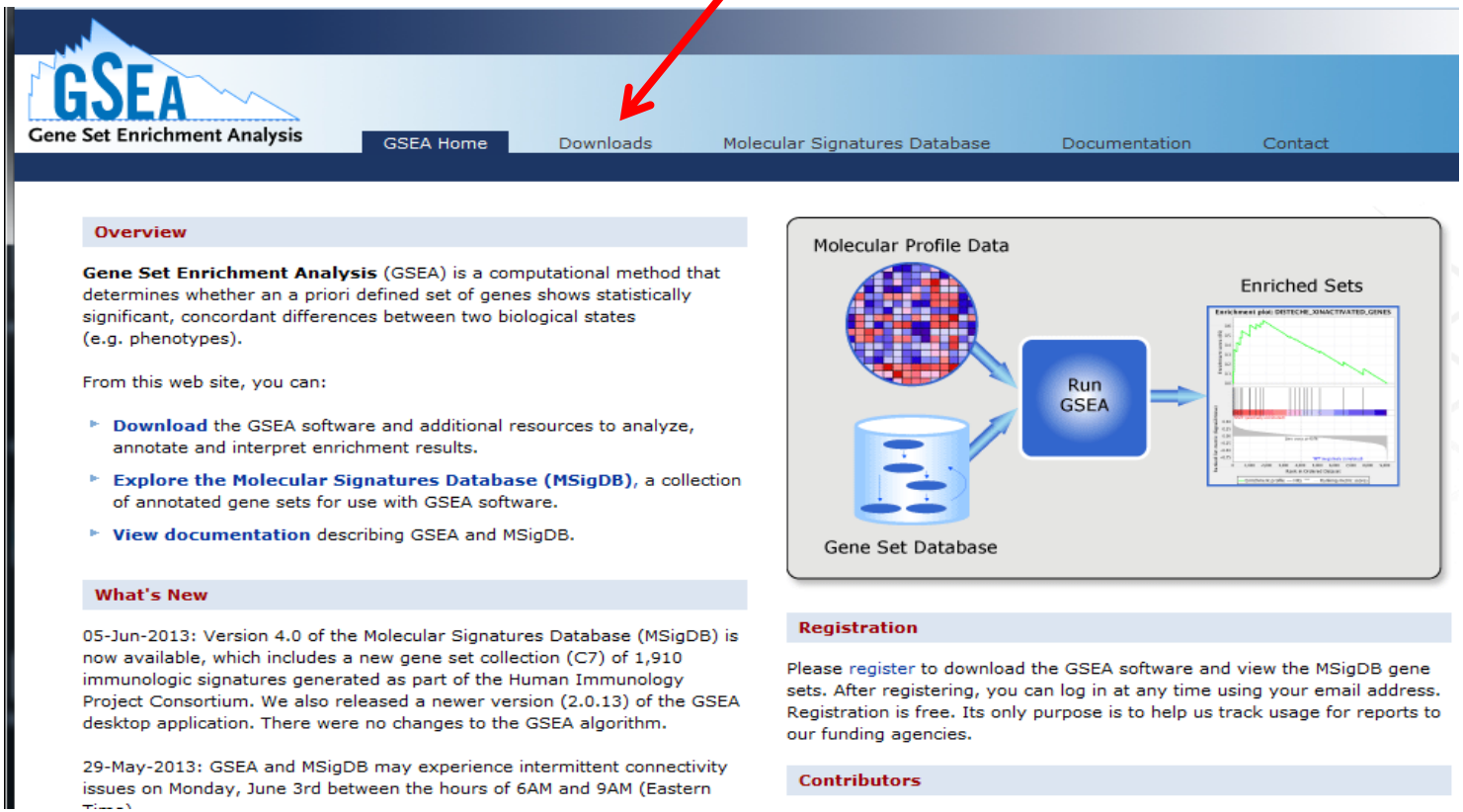

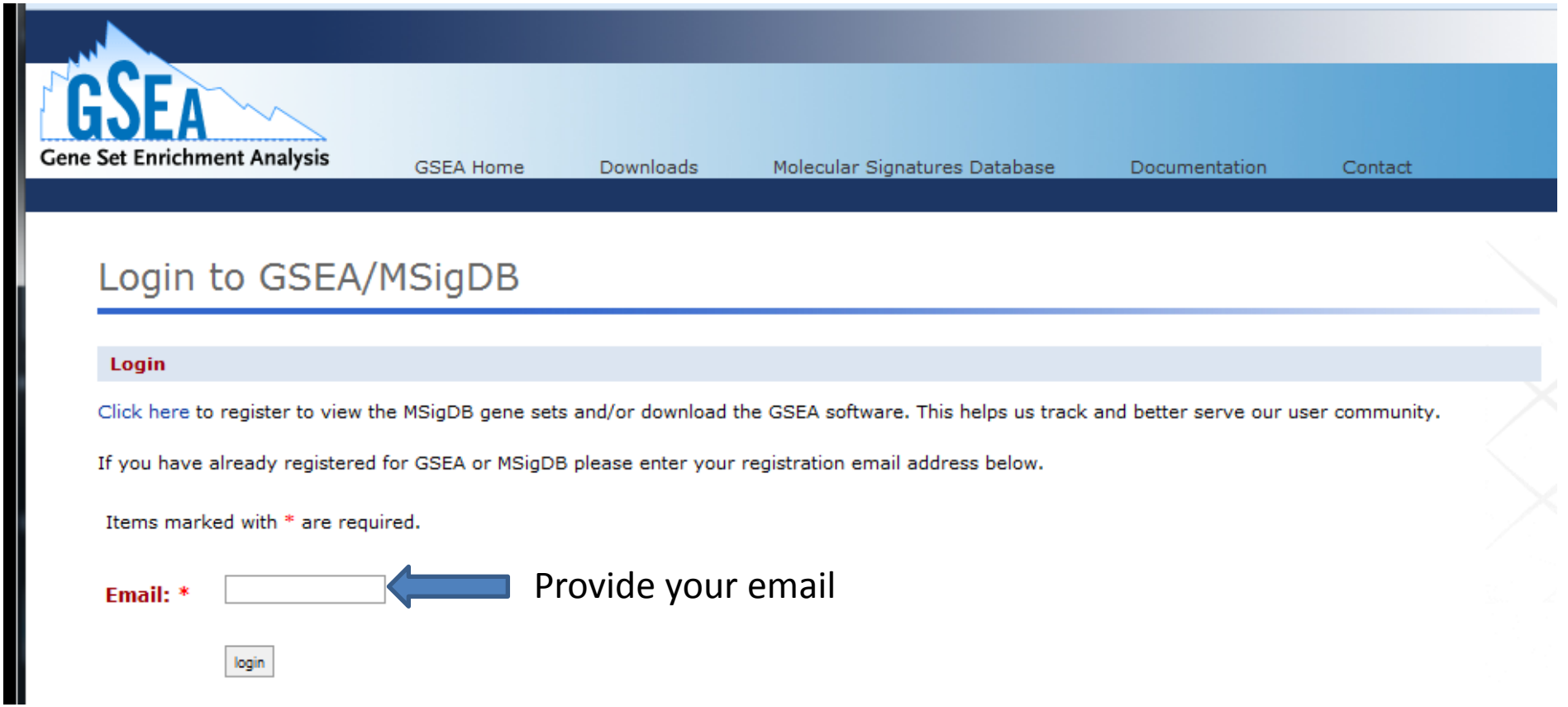
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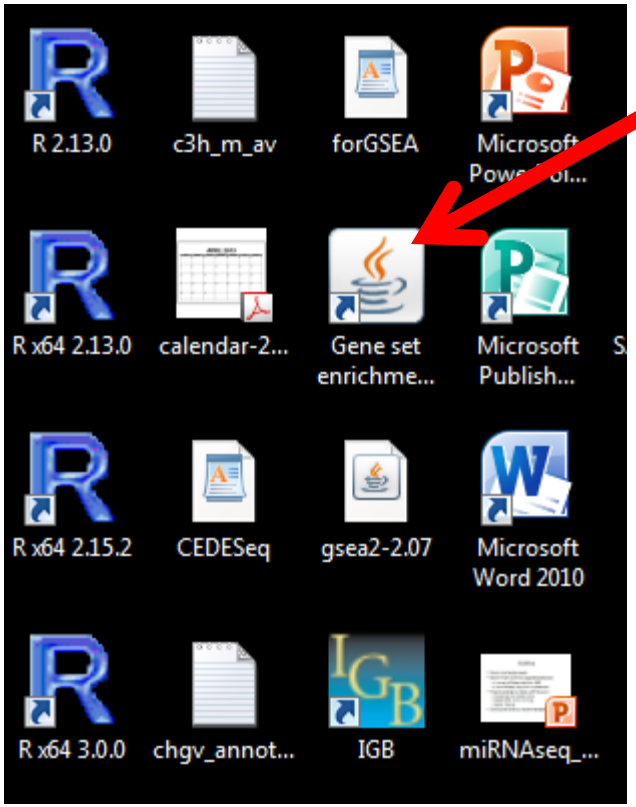


This is the icon you need to click to launch

Step 1

Step 2

Step 3

GSEA v2.0.12 (Gene set enrichment analysis -- Broad Institute)

File Options Downloads Tools Help

Steps in GSEA analysis

Load data

Run GSEA

Leading edge analysis

Gene set tools

Chip2Chip mapping

Browse MSigDB

Analysis history

GSEA reports

Processes: click 'status' field for results

Name	Status

Show results folder

Home | Load data ×

Steps in GSEA

1. What you need for GSEA

- Expression data set
- Phenotype annotation
- Gene sets – use MSigDB or your own gene sets

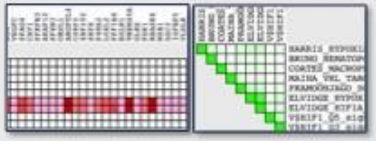
2. Run GSEA

- Start with default parameters
- If you want to collapse probes to genes, specify chip platform

3. View results

4. Leading edge analysis

- Leading edge finds genes driving enrichment results



Gene Set Tools

Chip2Chip mapping

- Convert gene sets between platforms

Chip2Chip mapping

Explore MSigDB gene sets

- Search the database of thousands of gene sets
- Browse the gene sets by name
- Find overlapping gene sets
- Export gene sets

Browse MSigDB

See also

- MSigDB online tools at: www.broadinstitute.org/msigdb

Getting Help

GSEA web site:
www.broadinstitute.org/gsea

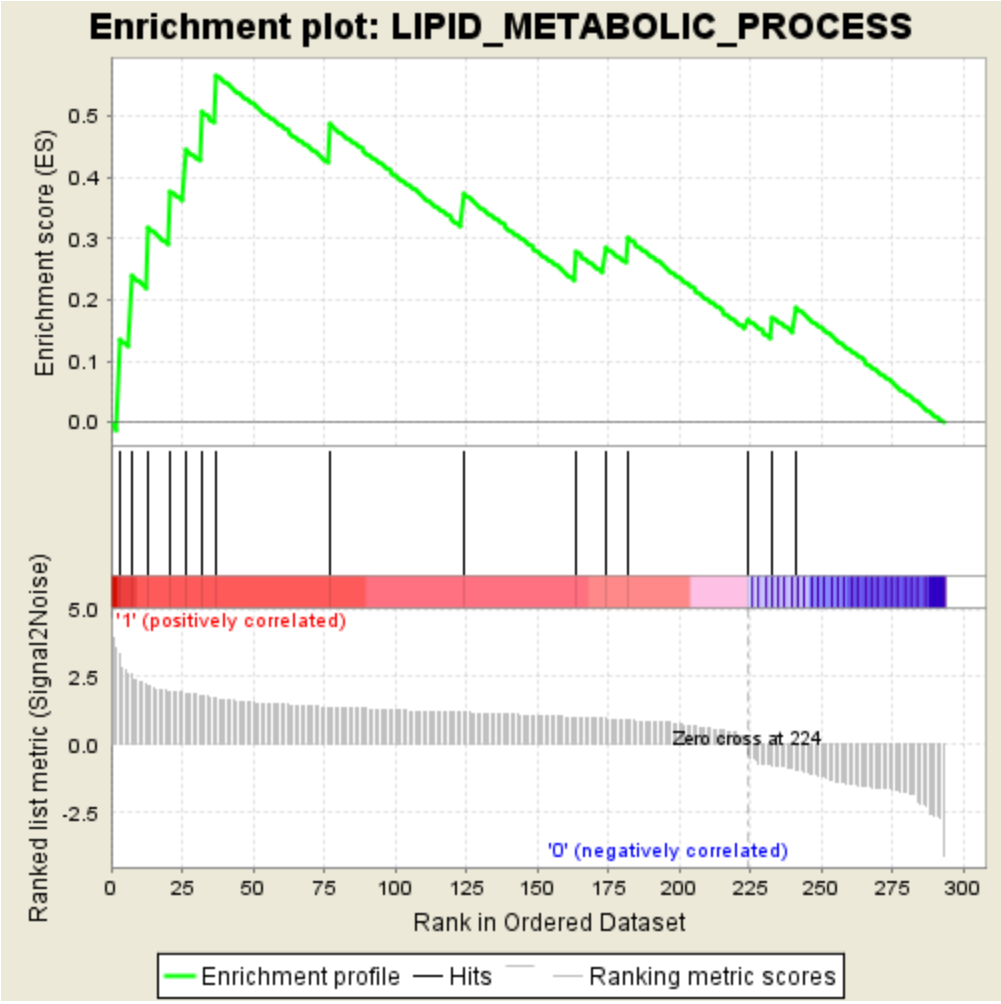
GSEA documentation:
www.broadinstitute.org/gsea/wiki

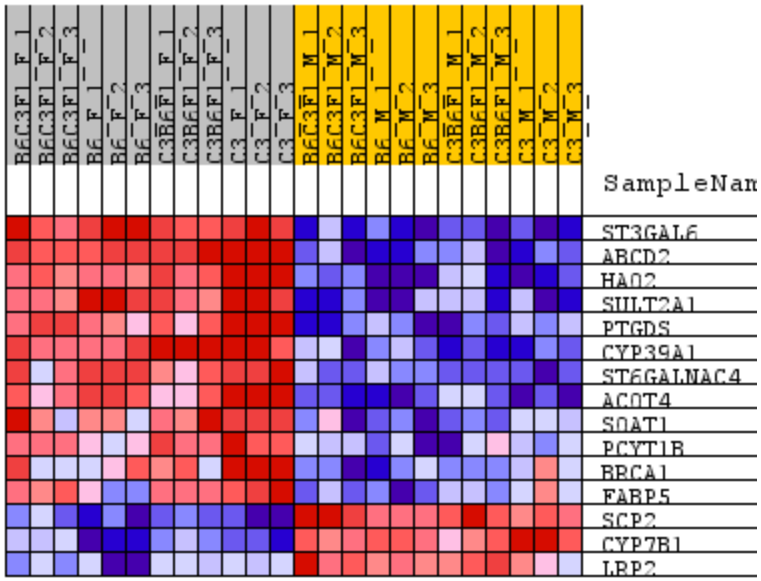
Email the GSEA team:
gsea@broadinstitute.org

BROAD INSTITUTE

4:03:18 PM | 6510 [INFO] Made Vdb dir: C:\Users\li11\gsea_home\output\jul11 | 14M of 61M

Lipid metabolism enriched -- GSEA





Genes involved (GSEA – cont.)

PROBE	GENE SYMBOL	GENE_TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
ST3GAL6	ST3GAL6 Entrez, Source	ST3 beta-galactoside alpha-2,3-sialyltransferase 6	3	3.348	0.1357	Yes
ABCD2	ABCD2 Entrez, Source	ATP-binding cassette, sub-family D (ALD), member 2	7	2.603	0.2389	Yes
HAO2	HAO2 Entrez, Source	hydroxyacid oxidase 2 (long chain)	13	2.192	0.3169	Yes
SULT2A1	SULT2A1 Entrez, Source	sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1	21	1.965	0.3777	Yes
PTGDS	PTGDS Entrez, Source	prostaglandin D2 synthase 21kDa (brain)	26	1.886	0.4459	Yes
CYP39A1	CYP39A1 Entrez, Source	cytochrome P450, family 39, subfamily A, polypeptide 1	32	1.783	0.5060	Yes
ST6GALNAC4	ST6GALNAC4 Entrez, Source	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 4	37	1.693	0.5658	Yes
ACOT4	ACOT4 Entrez, Source	acyl-CoA thioesterase 4	77	1.374	0.4861	No
SOAT1	SOAT1 Entrez, Source	sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase) 1	124	1.179	0.3728	No
PCYT1B	PCYT1B Entrez, Source	phosphate cytidylyltransferase 1, choline, beta	164	1.006	0.2770	No
BRCA1	BRCA1 Entrez, Source	breast cancer 1, early onset	174	0.946	0.2862	No
FABP5	FABP5 Entrez, Source	fatty acid binding protein 5 (psoriasis-associated)	182	0.899	0.3004	No
SCP2	SCP2 Entrez, Source	sterol carrier protein 2	224	-0.319	0.1674	No
CYP7B1	CYP7B1 Entrez, Source	cytochrome P450, family 7, subfamily B, polypeptide 1	233	-0.761	0.1720	No
LRP2	LRP2 Entrez, Source	low density lipoprotein-related protein 2	241	-0.901	0.1864	No

Some caveat in pathway analysis

- Concerns with GO based pathway analysis
  - Ignore GO hierarchy – treat each term independently
  - Ignore gene (expression) level/rank
  - Ignore correlation – assuming genes (in a category) are uncorrelated
  - Sampling over genes (observation)
  - All these make *p* values quite unclear
- Facts on the non-parametric approach
  - *Pros*
    - Relax on “strong assumption”
    - Customized gene set is allowed
  - *Cons*
    - Computationally intensive
    - Some concerns on the statistical power

The universe matters

- It is important to choose the universe correctly

Case 1: universe is all genes in the genome

	DEGs	Non-DEGs	Total
In GO	10	30	40
Not in GO	390	3570	3960
Total	400	3600	4000

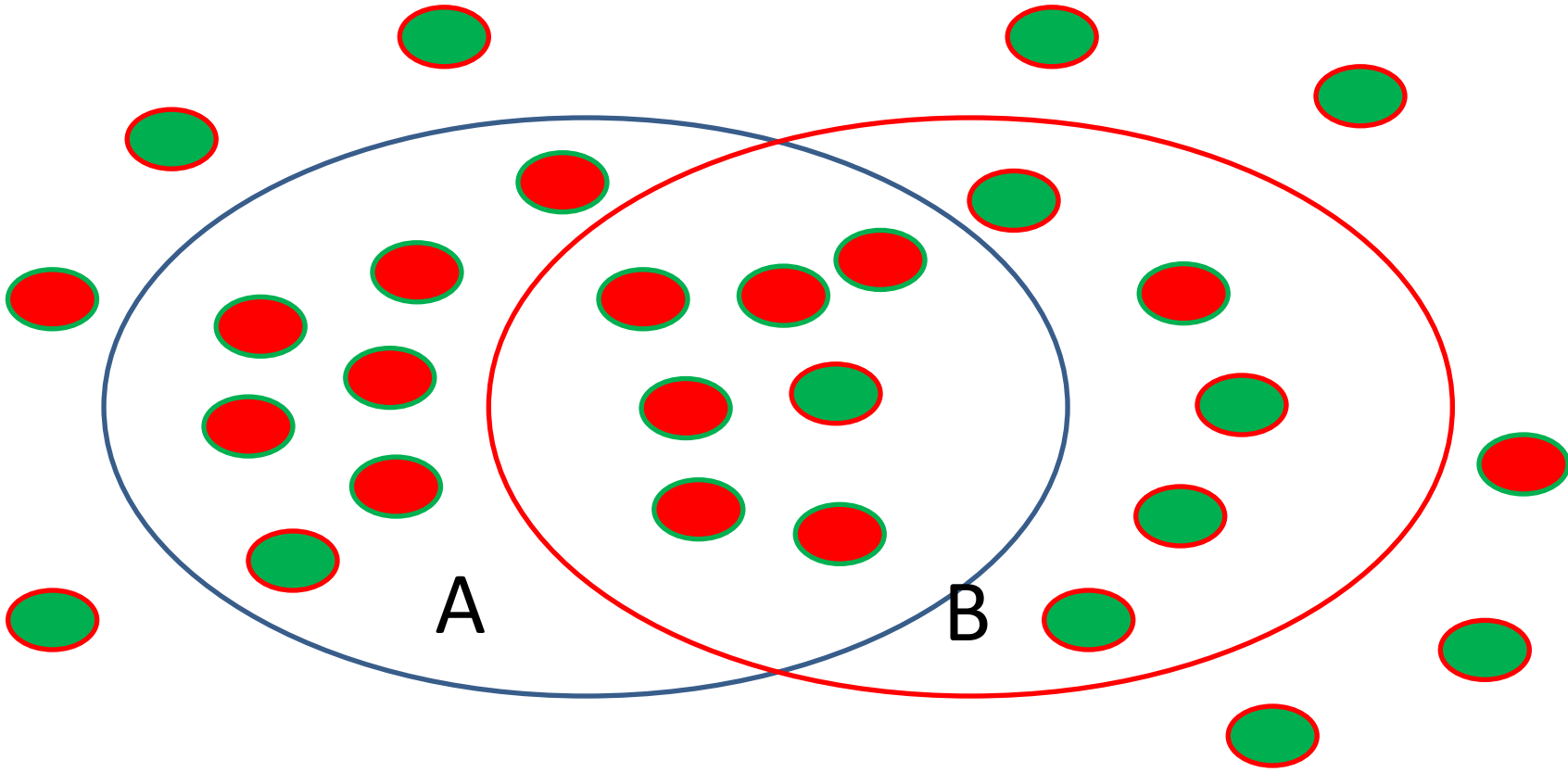
p=0.049

Case 2: universe is only expressed genes

	DEGs	Non-DEGs	Total
In GO	10	30	40
Not in GO	390	570	960
Total	400	600	1000

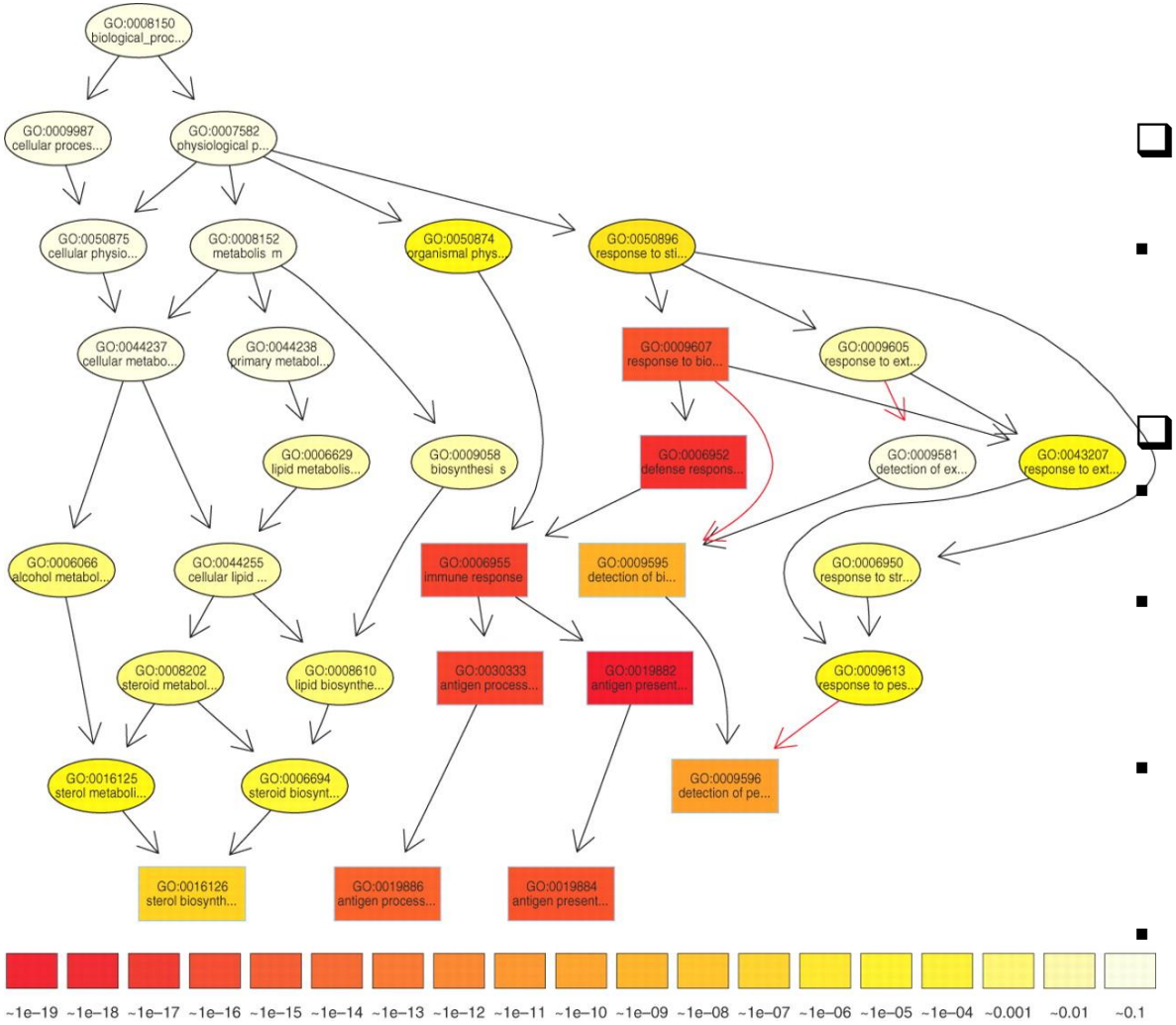
p=0.0048

Sets are overlapped



Set B is enriched only because of it overlap with set A

The subgraph induced by the 10 most significant GO terms identified by a current state-of-the-art method for scoring GO terms for enrichment.



❑ TopGO's elimination algorithm

- Test the leaf sets first. If significant, remove its "genes" before testing its ancestor sets

❑ TopGO's weight algorithm

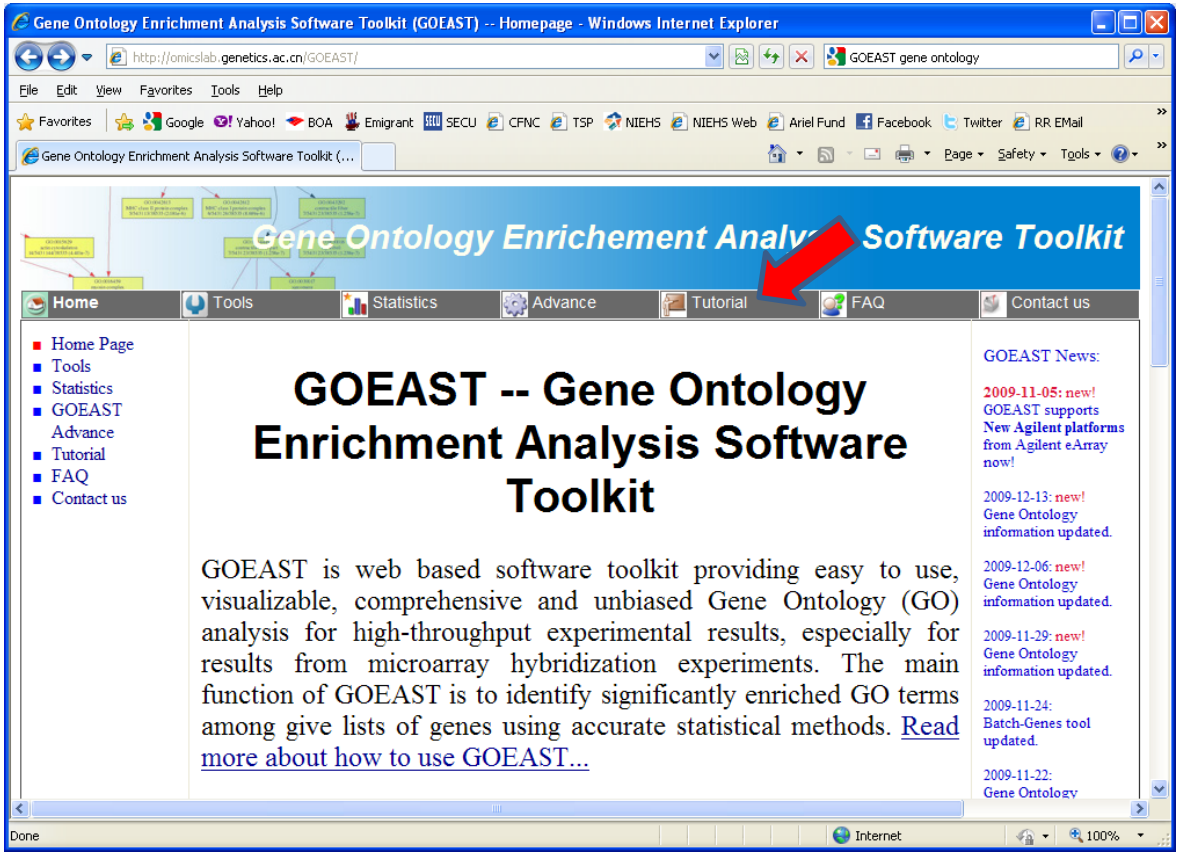
- The genes are weighted by their relevance in the significant nodes.
- The enrichment score of a parent (gene node u) is compared with the scores of its children.
- Children with a better score than u represent the interesting genes better. Therefore, their significance is increased
- Children with a lower score than u have their significance reduced.

Alexa A et al. Bioinformatics 2006;22:1600-1607

Bioinformatics

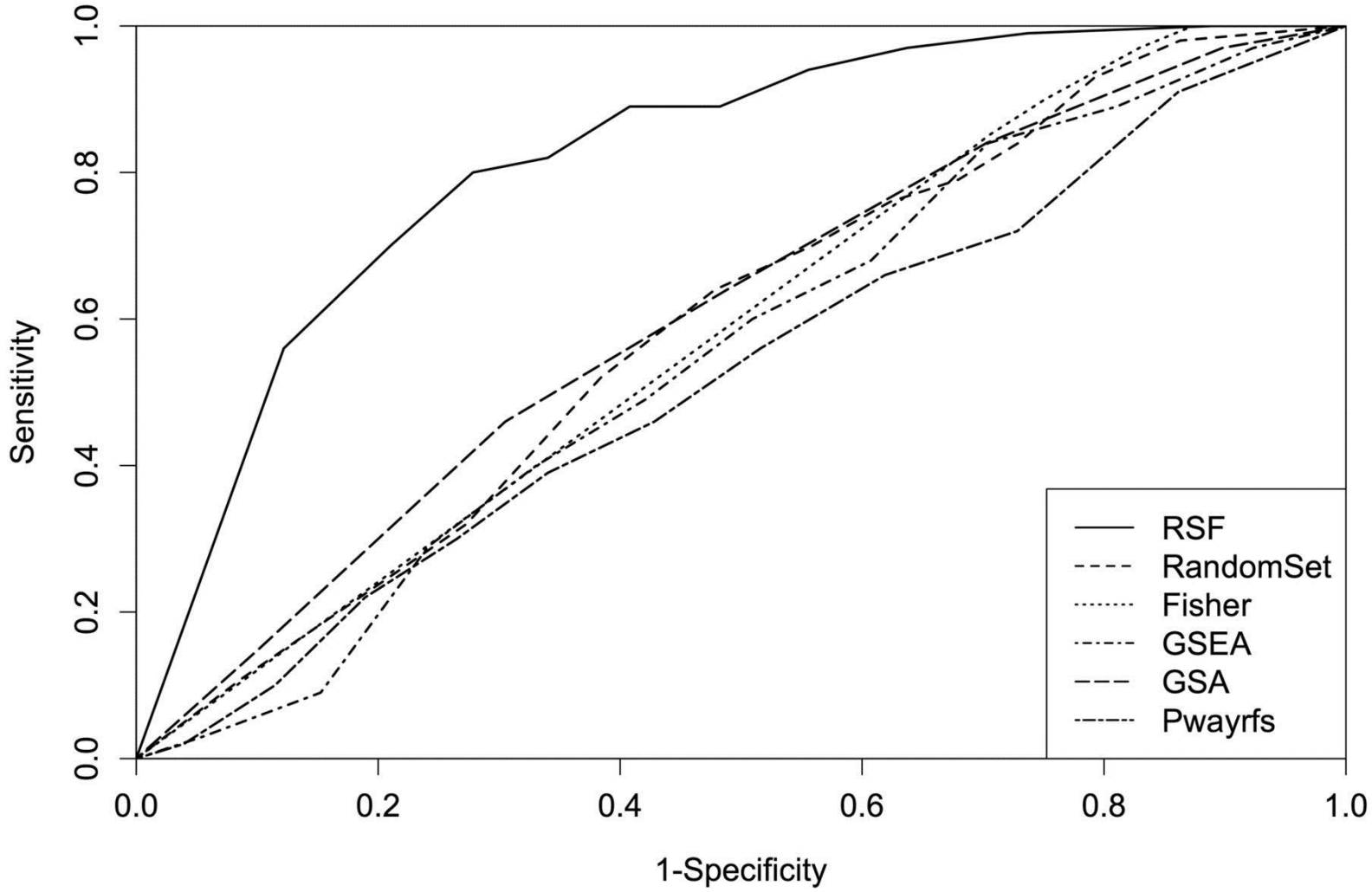
GOEAST – modification incorporated

Open web browser and navigate to: http://omicslab.genetics.ac.cn/GOEAST/



At the header tool bar, click on Tutorial

Comparison of performances of RSF, random-set, Fisher's exact test, GSEA, GSA and Pwayrfsurvival using simulated expression data.



Chen X , and Ishwaran H Bioinformatics 2013;29:99-105

Bioinformatics

GAzer

Gene Set Analyzer

Gene Set Analysis

Software and resources for inference on gene sets in microarray studies

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National Institute of Allergy and Infectious Diseases (NIAID), NIH

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GSEA

Gene Set Enrichment Analysis

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KEGG

Kyoto Encyclopedia of Genes and Genomes

- http://www-stat.stanford.edu/~tibs/GSA/
- http://www.netsci.org/Resources/Software/Bioinform/pathwayanalysis.html
- http://www.broadinstitute.org/gsea/index.jsp
- http://david.abcc.ncifcrf.gov/
- http://www.biocarta.com/
- http://web.expasy.org/pathways/
- http://www.genmapp.org/
- http://www.genome.jp/kegg/
- http://www.ingenuity.com/
- http://www.genego.com/metacore.php
- http://www.geneontology.org/
- *http://omicslab.genetics.ac.cn/**GOEAST**/tutorial.php*
- *http://expressome.kobic.re.kr/GAzer/document.jsp*
- *http://www.biobase-international.com/products*
- *http://jaspar.genereg.net/*

Pathway analysis summary

- Two major statistics approaches
  - Parametric vs. non-parametrics
- Three major databases
  - Gene Ontology
  - KEGG
  - Transfac & transpath
- A few popular applications
  - DAVID (free online)
  - GSEA (online and desktop java application)
  - GSA (R –package)
  - Ingenuity Pathway Analysis (licensed based)
  - Biobase/JASPER (license based)
  - GeneGO (license based)